

POLYCLONAL ANTIBODY UTILIZATION FOR DETECTION OF JEMBRANA ANTIGEN AT BALI CATTLE IN WEST SUMATRA PROVINCE

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ABSTRACT

The aims of this study were to detect Jembrana antigen with polyclonal antibody and to describe antigen distribution in the Bali cattle organ that positively infected with Jembrana disease. Spleens, lungs, and livers were harvested from 10 naturally infected Bali cattle whose infection was confirmed through positive Polymerase Chain Reaction (PCR) result for Jembrana virus. Immunohistochemistry test was performed using polyclonal antibodies produced in rabbits. The results showed that cells infected by Jembrana viruses displayed positive reaction with a reddish brown color. Immunohistochemistry methods using polyclonal antibodies can detect Jembrana antigens in the spleen, liver, and lung with the highest average detection score ($P < 0.05$) was found in spleen, followed by liver and lungs. There was significant difference in the distribution of Jembrana antigens between the spleen, liver, and lungs with spleen having the highest antigen density.

Key words: immunohistochemistry, Jembrana, polyclonal antibody

ABSTRAK

Penelitian ini bertujuan mendeteksi antigen jembrana dengan menggunakan poliklonal antibodi dan mengetahui distribusi antigen jembrana pada jaringan organ limpa, hati, dan paru-paru sapi bali yang terinfeksi virus jembrana. Jaringan organ sapi bali yang terinfeksi secara alami di lapangan sebanyak 10 ekor, diambil organ limpa, paru-paru, dan hati yang telah dilakukan uji Polymerase Chain Reaction (PCR). Uji imunohistokimia dilakukan dengan menggunakan antibodi poliklonal yang diproduksi pada kelinci. Hasil uji imunohistokimia terlihat sel-sel yang terinfeksi oleh virus jembrana menunjukkan reaksi positif dengan warna coklat kemerahan. Pada pengujian metode imunohistokimia dengan menggunakan antibodi poliklonal dapat mendeteksi antigen jembrana dalam organ limpa, hati, dan paru-paru. Rata-rata skor deteksi antigen jembrana ($P < 0,05$) terbanyak ditemukan pada organ limpa, hati, dan paru. Ada perbedaan yang nyata distribusi antigen jembrana di antara organ limpa, hati dan paru-paru. Pada organ limpa terdeteksi antigen virus jembrana lebih banyak dibandingkan dengan organ hati dan paru-paru.

Kata kunci: imunohistokimia, Jembrana, antibodi poliklonal

INTRODUCTION

Bali cattle are one of the important native Indonesian cattle and widely distributed throughout the country. Bali cattle have high adaptability to unfavorable environments (Masudana, 1990) and could breed well in various breeding environments throughout Indonesia. Despite of their advantage, it also has certain shortcoming such as relatively small body size, low milk production resulting in slow calf growth, and is the most vulnerable to Jembrana disease (Soeharsono *et al.*, 1990). The latter is caused by genetic predisposition. Jembrana is an infectious disease in cattle which specifically attacks Bali cattle (*Bos javanicus*). This disease was first identified in Jembrana Regency, Bali Province (Indonesia), which is caused by Jembrana virus, from the genus Lentivirus of Retroviridae family (Kusumawati *et al.*, 2015).

Lentiviruses have a rough round shape with a diameter of 80-100 nm and are enveloped by lipid bilayer. Jembrana virus is sensitive to heat, detergent, and formaldehyde (Goff, 2001). Jembrana virus has negative ssRNA genetic material. Its genome consists of 7,732 nucleotide bases. Similar to other lentivirus genomes, it has three main genes or structural genes that encode important proteins such as gag, pol, and

env, as well as long terminal repeats (LTR) that are distinct to retroviruses (Chadwick *et al.*, 1995).

Jembrana disease is endemic in Indonesian, especially in Java, Sumatra, and Kalimantan (Hartaningsih *et al.*, 1993). Jembrana disease first appeared and became epidemic in West Sumatra in April 1992 at Baringin Sakti (Timpeh II) village in Sawahlunto Sijunjung District (Hartaningsih *et al.*, 1993; Wilcox *et al.*, 1997), then in 2014 at Dharmasraya, and in 2015 at Agam district (Miswati, 2016), finally the latest was at January 2019 which still caused significant cattle death due to Jembrana.

Among infected Bali cattle, the mortality rate could reach 21% (Soeharsono *et al.*, 1990; Soesanto *et al.*, 1990). This finding is supported by evidence obtained in several epidemiological studies (Soeharsono *et al.*, 1995; Chadwick *et al.*, 1998). In fatal infections, death occurs within 1 to 2 weeks after the onset of infection and correlates with multiple organ failure (Wilcox, 1995; Wilcox *et al.*, 1997).

Jembrana disease is commonly diagnosed based on clinical symptoms and immunology diagnostic techniques. Clinical diagnosis is difficult because of its similarity to symptoms of other diseases, and even then symptoms only appear in acute Jembrana disease. Routine Jembrana virus identification is carried out in

the laboratory using Polymerase Chain Reaction (PCR). This test can detect viral nucleic acid in acute phase (Stewart *et al.*, 2015) and can detect viruses in live animals, however due to the high cost of the equipments and reagents, it is difficult to be adopted by most laboratories in Indonesia (Astawa *et al.*, 2006). Immunohistochemistry techniques (IHC) has already been used to detect Jembrana virus antigens by utilizing Jembrana monoclonal antibodies (MoAb) (Dharma, 2002), this test can detect and determine the distribution of antigens in animal organs infected with Jembrana virus. Compared to PCR assay, immunohistochemistry techniques is cheaper, but considering the limited availability of MoAb Jembrana, it is rarely used. Therefore, it is necessary to have an alternative test such as IHC testing using Jembrana polyclonal which is easier to obtain and cheaper to use. IHC techniques are useful for identification, localization, and characterization of a particular antigen, as well as diagnosing. The aims of this study to detect Jembrana antigen with polyclonal antibody and to describe antigen distribution in the Bali cattle organ that infected with Jembrana disease naturally.

MATERIALS AND METHODS

The samples were tissue organs (spleen, liver, and lung) from 10 Bali cattle infected with Jembrana virus from Regency/City in Bukittinggi Veterinary Center (Lima Puluh Kota, Padang Pariaman, Pesisir Selatan, Dharmasraya, West Sumatra Province). Sampling was conducted from January 2018 to March 2019. All new cases within those periods were included in the experiment.

Research Procedure

Samples of spleen, lung and liver organs were extracted from necropsy of Bali cattle infected with Jembrana virus which was previously confirmed positive for Jembrana using PCR method.

Polyclonal Antibody Production

Three local rabbits used in this study were adapted for 2 weeks and then vaccinated with Jembrana vaccine. The vaccine was produced by Pusvetma Surabaya. Vaccine was given at week I, booster at week II, III and IV with 1 dose (3 mL) each and injected in two different places. Blood serum was collected at week 5. The collected serum were used in immunohistochemistry tests on Jembrana positive organ samples that were confirmed by PCR.

Immunohistochemistry

Jembrana virus antigen detection using immunohistochemistry staining techniques was based on modified two-step Polymer labeling method (Ramos-Vara and Miller 2006), using a polyvalent (universal) reagent detection system (RealTM EnvisionTM (K5007 DAKO®, DENMARK). Tissue sections in 4-5 µm-thick paraffin were placed on glass objects coated with poly-L-lysine. The slides were then deparaffinized in xylol, rehydrated in multilevel

alcohol, washed with Phosphate Buffered Saline Tween 20 (PBST), given 3% H₂O₂ dissolved in methanol to block endogenous peroxidase, and given normal goat serum to eliminate nonspecific reactions.

The presence of Jembrana virus antigens in tissues was detected using serum (1:1000) collected from rabbits. The slides were stained with 3-3 diaminobenzidine (DAB) chromogen and as the counterstain we used Mayer Hematoxylin to create bluish color in the tissue. Then the slide was closed with a cover-slide which was previously mounted with entellan, and then observed under light microscope at 20x magnification.

Immunohistochemistry Observation

IHC staining on each part of spleen, liver and lung are observed under light microscope at five fields with 200 times magnification, we found positive reactions to Jembrana virus antigens that were characterized by brownish appearance in the cells. Distribution of antigens in each field of view was calculated and scored according to Suja *et al.* (2009), with following criteria: (-) = no Jembrana antigen found, (+) = Jembrana antigen found in 1-30%, (++) = Jembrana antigen found in 30-60%, and (+++) = Jembrana antigen found in 60-100%.

Data Analysis

Scoring data of antigen distribution were analysed using one way analysis of variance.

RESULTS AND DISCUSSION

Identification of Jembrana Viruses

Molecular techniques make it possible to accurately identify Jembrana virus at DNA level using PCR method. A PCR test on 10 samples of Bali cattle organs that died on the field showed that all 10 samples were positive for Jembrana virus. Jembrana disease can be accurately diagnosed by PCR, ELISA, and Immunoblotting, however, in this case the diagnosis is considered more accurate by using PCR. This is consistent with Barboni *et al.* (2001), who found that serological tests were only able to detect 5 out of 10 samples (50% accuracy). However, PCR technique was 100% accurate, with detection rate of 10 positive out of 10 samples. Based on the molecular data retrieved, precautions can be taken in areas where Bali cattle have been fatally infected with Jembrana virus. Figure 1 depicted the results of PCR on Jembrana virus reading and analysis by UV trans-illuminator (Gel Documentation). The DNA molecule length of the Jembrana virus PCR product is 365 bp. All samples positive for Jembrana virus were used for IHC testing to see the extent of the spread on the Bali cattle organs in West Sumatra Province.

Analysis of Jembrana Virus Protein Profile with SDS-PAGE and Immunoblotting

Jembrana virus protein in rabbit serum that has been identified was then analyzed for its protein profile. The result of the 26 kDa protein profile analysis was

visualized through the transfer of protein molecules from gel matrix to nitrocellulose-dense membranes and shown in Figure 2. According to Bollag and McCormick (1991), protein profile was analyzed by separating the viral protein from other proteins or other molecules based on their size, solubility, charge, and bond affinity. According to Wilcox *et al.* (1997), Jembrana virus has various types of proteins such as p100, p45, p33, p16, and major protein p26 which cross-react with antigens and antibodies of BIV virus (bovine immunodeficiency virus). From immunoblotting, it has been confirmed that the serum formed in the rabbit's body is a polyclonal serum that will be used in immunohistochemistry testing.

Detection of Jembrana Antigen in a Tissue

Evaluation of all samples which shows positive antigens reaction was displayed as brown color (Figure 3). Antigens are deposited in the cytoplasm of cells from organs and immune cells such as lymphocytes, macrophages, buffer cells and other cells with different

numbers and patterns of spread. According to Dharma (2010), immunohistochemistry staining techniques are known to identify Jembrana virus antigens in organ tissues, especially spleen.

The highest average Jembrana antigen score was found in the spleen, followed by liver and lungs with considerable difference (Table 1). This was in accordance with Chadwick *et al.* (1998) that described spleen as the organ that contains the largest amount of cells infected by Jembrana virus. According to Dharma *et al.* (1991) cells infected with Jembrana virus in the spleen are located in parafollicles, which indicates that they are T lymphocytes. Spleen tissue is the most frequent site containing Jembrana antigen, compared with liver and lung tissues. Jembrana antigen granules are located in the cytoplasm of lymphocytes in the white pulp.

In consideration for the ease of sample collection and sufficiently large amount of viral antigen in the spleen, spleen is the preferred sample to be collected compared to lungs and liver for disease testing.

Table 1. Average distribution of Jembrana antigens in organ tissues

| No | Organ tissues | Distribution of antigen |
|----|---------------|-------------------------|
| 1 | Spleen | 2.6 ± 0.52^a |
| 2 | Lung | 1.7 ± 0.48^b |
| 3 | Liver | 1.6 ± 0.52^b |

^{a, b} Different superscripts within the same column indicate significantly different

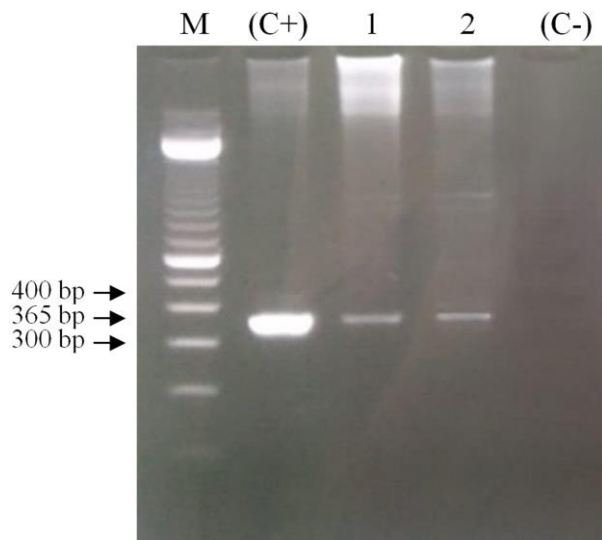


Figure 1. RT-PCR results of Jembrana virus. M= Marker, 1-2= Sample, (C+)= Positive control, (C-)= Negative control

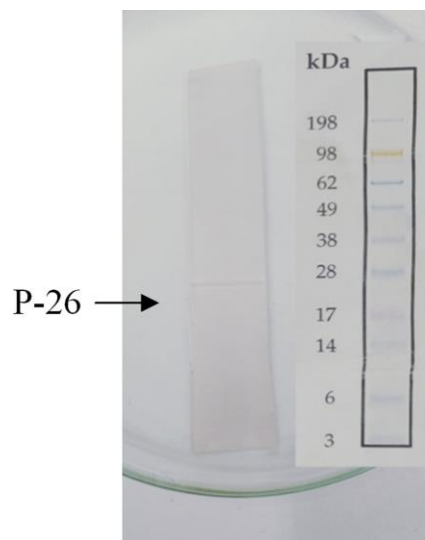


Figure 2. Jembrana virus protein profile (→ 26 kDa)

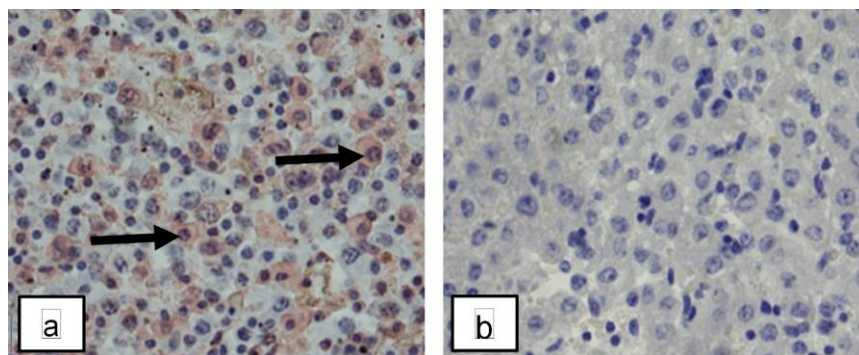


Figure 3. Jembrana virus antigens (→) in spleen. A= Positive b= Negative

The difference in the shape of antigens indicates viral development, replication and deposition of antigen that represent density of virus proteins. In addition, the difference in the shape of antigen is thought to be related to viral content in each tissue; in areas with dense antigens, we suspect a higher amount of virus compared to granular ones while granular-shaped antigens have higher viral contents than tissues with point-shaped antigen appearance.

CONCLUSION

Based on immunohistochemistry tests using polyclonal antibodies, the highest amount of Jembrana antigens was found in spleen, followed by liver and lungs. There was a significant difference between distribution of antigens in the spleen, liver, and lung on Bali cattle in West Sumatra.

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